Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(8): 124-131

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The Potential Protective Role of Quercetin against Nano-Rich Diesel Exhaust Particles induce Hepatic Apoptosis in Albino Rat Fetuses

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ABSTRACT

Supplementation with vegetables and fruits derived products plays a unique role in the protection against various diseases as it has many flavonoids that provide several health benefits. Exposure to nano-rich diesel exhaust particles (NRDEPs) especially during developmental stages may be induce oxidative damage and trigger apoptosis in different fetal organs. So, the present study aims to evaluate the hepatic protective effect of quercetin against toxicity of NRDEPs in albino rat fetuses. A total of forty timed-pregnant albino rats were randomly assigned to four different groups. The first group served as negative control and received normal saline, dams of the second group received 100 mg/kg of quercetin, and those of the third group received 20 mg/kg of NRDEPs, the fourth group received 100 mg/ kg of quercetin two hours before NRDEPs exposure. All dams were received their corresponding doses from 5th day of gestation till to 20th day. The animals were received caesarean section at end of gestation and fetal liver was removed for immunohistochemical examination together with biochemical and molecular studies. Our results indicated that maternal pretreatment with quercetin significantly ameliorated the oxidant / antioxidant disturbance (MDA, GSH, GST and PON) induced by NRDEPs, down regulated the apoptotic genes (Bax and caspase-3) expression and reduced DNA fragmentation. In conclusion, quercetin could suppress the oxidative stress and apoptosis induced by nano-rich diesel exhaust particles in fetal liver without side effects. We suggest that quercetin may be useful in combating cytotoxicity caused by NRDEPs.

Keywords: Diesel exhaust particles; Nanoparticles; Quercetin; Apoptosis; Fetal liver

Abbreviations: Bax: Bcl-2–associated X protein; Bcl-2: B-cell lymphoma 2; Bcl-XL: B-cell lymphoma-extra-large; CNTs: Carbon Nanotubes; DE: Diesel Exhaust; NP: Nanoparticles; NRDEPs: Nano-Rich diesel Exhaust Particles; PAHs, Polycyclic Aromatic Hydrocarbons; PM, Particulate Matter; ROS, Reactive oxygen species

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INTRODUCTION

The diesel fuel combustion in engines produces microscopic particles known as diesel exhaust particles (DEPs), which constitutes the foremost part of particulate matter, are characterized by a carbonic mixture composed of different chemicals with different sizes and physical proprieties such as polycyclic aromatic hydrocarbons (PAHs), quinones, aldehydes and toxic heavy metals [1].

Nano-rich diesel exhaust particles (NRDEPs) are notably rich in toxic heavy metals as Al, B, Ba, Cr, Cu, Fe, Na, Ni and Zn [2], approximately spherical shape nanoparticles (20-60 nm), carbon nanotubes (CNTs) and carbon nano-chains [3].

Since, nano-rich diesel exhaust particles can exhibit undesirable harmful effects on the fetuses during the very sensitive pregnancy period [4], the potential effects of NRDEPs exposure to the pregnant women are of interest to society and regulatory agencies.

Experimental studies showed that the exposure to dust storm fine particles in rats provided convincing evidence for a causative role for particulate matter in oxidative damage in the liver [5-7]. Other studies showed the effects of wood smoke particulate matter and carbon black in causing hepatotoxicity by inducing oxidative stress [7] and the effects of toxic metals, existing as an organometallic complex, in producing increased lipid peroxidation in liver [8].

In fact, the developing mammalian liver is highly susceptible to chemicals and nanoparticles exposure as compared to the adult. So, exposures to NRDEPs during critical periods of development are suspected to induce lasting adverse effects in early development and later in life [9].

There is strong evidence from experimental studies that NRDEPs can cross the placental barrier and reach the fetus [10,11]. Also, the hepatotoxic associations of high level prenatal and early childhood exposure to certain nanoparticles are not well established so, the potential toxic effects on fetal liver associated with nano-rich diesel exhaust particles are challenging to interpret.

Actually, the best solution to alleviate the developmental and pathological changes occurred due to oxidative stress and/or inflammation is to use some neutral, safe and available antioxidants and/or anti-inflammatory compounds.

Quercetin has various protective and ameliorative functions including, metal chelator, free radical's scavenger, anti-inflammatory and may prevent oxidative stress, cell injury and apoptosis [12], so we designed this study to investigate the suppressive roles of quercetin against the fetal hepatotoxicity induced by nano-rich diesel exhaust particles.

EXPERIMENTAL SECTION

Chemicals Used

Quercetin (2,3′,4′,5,7-pentahydroxyflavone), other chemicals and substrates were obtained from Sigma Chemical Company (U.S.A.). The NRDEPs were obtained from the Egyptian Transport Network.

Experimental Design

A total of forty timed-pregnant albino rats (Rattus norvegicus) were randomly assigned to four different groups. The control group (G1): received normal saline and the quercetin group (G2): received 100 mg/kg of quercetin orally. The intoxicated one (G3): received 20 mg/kg of NRDEPs with intratracheal instillation and the pretreated group (G4): received 100 mg/ kg of quercetin orally, two hours prior to NRDEPs exposure and all dames were received their corresponding doses from 5th days of gestation till to 20th days. Our protocol was approved by The Institutional Animal Care and Use Committee (IACUC) at Cairo University (CU.1.S.74.17).

Specimen

By the end of gestation, the dams were anesthetized and received caesarean section. The liver specimens were removed immediately from the fetus, washed, dried and fixed by formalin for immunohistochemistry. Other liver tissues were kept frozen at -80°C for biochemical analysis, comet assay and real-time polymerase chain reaction (Real-Time PCR).

Biochemical Analysis

Preparation of the tissue fraction

Fetal liver tissues were homogenized in normal saline, then the homogenate was centrifuged and the resultant supernatant was used for biochemical analysis. The protein concentration was measured calorimetrically by the method adopted by [13].

Determination of lipid peroxidation level

Malondialdehyde (MDA) as an end product of lipid peroxidation, which can react with thiobarbituric acid in acidic medium to produce thiobarbituric acid reactive complex displaying an absorption maximum at 534 nm [14].

Determination of reduced glutathione (GSH) content

GSH determination was based on the production of a yellow colored complex in a reaction between (5,5-dithiobis(-2-nitrobenzoic acid)) and sulphydryl compounds which has a specific absorption at 412 nm [15].

Determination of glutathione-S-transferase (GST) activity

The activity of GST was assayed spectrophotometrically at 25°C using 1-chloro-2, 4-dinitrobezene (CDNB) as a substrate following a previously published method [16].

Determination of paraoxonase (PON) activity

The activity of paraoxonase was determined spectrophotometrically and p-nitrophenyl acetate was used as a substrate, the activity of the enzyme was measured by the rise in the developed yellow colour during p-nitrophenol released as previously described [17].

Immunohistochemistry

Paraffin embedded liver tissues were cut into seven µm sections and rabbit Bax and caspase-3 antibodies were applied as a primary antibody. A biotinylated secondary antibody was added, then horse-radish peroxidase conjugated with streptavidin. As streptavidin has a great affinity to biotin, it binds to the position where primary antibody coated the background, once adding a chromatogen a brown colour appears [18], a light microscope with a digital camera were used for examination and images capture.

Comet assay

The alkaline comet assay (pH>13) was applied using microscope slides [19]. Fetal liver tissues were minced in Hank´s balanced salt solution (HBSS). Minced liver tissues were collected and centrifuged. Cell pellet was suspended in PBS-CMF. The slides (prepared one day before) were pre-coated with 200 µL of 1.5% normal melting point agarose. Then a mixture of 10 µL of cell suspension and 75 µL of 0.5% low melting point agarose was added. The covers were gently slid off and a third layer (80 µL of 1.0% low melting point agarose) was added to the slides. The covers were replaced and the slides were allowed to harden for 3–5 minutes and the slides were slowly immersed in lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base with pH 10. Freshly 1% Triton X-100 and 10% DMSO). The slides were removed from lysing solution and rinsed to remove salts and detergents. The slides were placed close together on horizontal gel electrophoresis (25 V and 300 mA for 40 min; JSB-30, USA). After the electrophoresis, slides were washed using neutralization buffer. Then the slides were fixed by 70 % ethanol and stained with aqueous ethidium bromide solution. DNA fragmentation was examined using fluorescence microscope (Lx 400, Labomed, USA) and determined by v1.5 of Comet Score.

Expression of apoptosis genes determination

Total RNA was extracted from the fetal liver tissues using RNeasy Mini Kit (Catalogue no.74104) and corresponding cDNA was synthesized using (RevertAid Reverse Transcriptase Thermo Fisher, Catalog number: K1622) according to manufacturer's instructions. The cDNA products were stored at −20◦C until to use. The primers for target and internal reference (β-actin) genes were obtained from (Metabion, Germany) and the sequences of these primers obtainable in Table 1. Real-time quantification was performed in the Stratagene MX3005P instrument using the Quantitect SYBR green PCR kit (Cat. No. 204141).,with total reaction volume as 25 µL containing approximately 12.5 µl 2x SYBR Green PCR Master Mix, 1 µl primers, 2 µl cDNA, and 8.5 µl of RNase Free Water. Negative and positive controls were used for the accuracy of results. Relative levels of target genes mRNA were calculated using the 2−ΔΔCt method [20].

Table 1: Oligonucleotide primers used in SYBR Green RT-PCR

Gene	Primer sequence (5'-3')	Accession Number	Reference
ß-actin	*F*-TCCTCCTGAGCGCAAGTACTCT	V01217	Banni et al. [21]
	R-GCTCAGTAACAGTCCGCCTAGAA		
BAX	*F*-CACCAGCTCTGAACAGATCATGA	RRU49729	Kinouchi [22]
	R-TCAGCCCATCTTCTTCCAGATGGT		
Caspase-3	*F*-AGTTGGACCCACCTTGTGAG	NM_012922.2.298	Shi et al. [23]
	R-AGTCTGCAGCTCCTCCACAT		

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Statistical analysis was performed using the analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc analysis to determine the differences between groups. Significance was set at probability $P \leq 0.05$ using IBM SPSS version 22 software package (SPSS, IBM, Chicago, IL, USA).

RESULT AND DISCUSSION

Despite broad maternal exposure, developmental health outcomes, mechanisms of action of nano-rich diesel exhaust particles (NRDEPs) and the best way to prevent their toxic effects have been subject to surprisingly few studies.

This study was specifically conducted to evaluate the protective effects of quercetin on the liver of albino rat fetuses maternally exposed to NRDEPs with mechanistic focus on oxidative stress, apoptosis and DNA damage.

Malondialdehyde (MDA) level

The fetal liver MDA level was significantly augmented ($P<0.05$) in both intoxicated and pretreated groups as compared with control one (Table 2), revealed a damaging effects on cell membrane structure and functions by the theory of lipid peroxidation.

Surely, NRDEPs can cross the placenta, and may reach the fetal organs [24], which leads to induce oxidative stress and apoptosis [25] due to their distinctive physicochemical structures as their small size with large reactive surface area are responsible for generating ROS and oxidative injury [26], or by their chemical composition such as poly aromatic hydrocarbon and their derivatives as well as transition metals [27].

The most reasonable causes, for NRDEPs to generate free radicals, are that they localize in mitochondria, where they induce major structural damage. This may contribute to oxidative stress and increased biological potency of NRDEPs is related to the content of redox cycling organic chemicals and their ability to damage mitochondria [28], or due to their reaction with H2O2 which causes DNA and cellular damage [29].

In addition, we can't neglect the associations between oxidative stress in terms of 8-oxoguanine and oxidation products of proteins and lipids, and the exposure to NRDEPs [30].

According to our results, maternal pretreatment with quercetin (100 mg/kg) attenuated lipid peroxidation in fetal liver maternally exposed to NRDEPs (Table 2).

The antioxidant property of quercetin is most often attributed to its hydroxyl groups, which relates with its electron donating capability [31], so quercetin prevents the free radical chain reaction progression through the mechanism of free radicals trapping at the interface of the membranes [32], or can chelate transition metal ions present in NRDEPs that may be responsible for generating free radicals [33], consequently, quercetin is able to inhabit lipid peroxidation [34].

Table 2: Effects of NRDEPs and pretreatment with quercetin on MDA and GSH levels, GST and PON activities in fetal liver tissues

	MDA (nmol/g tissue)	GSH (µM/g tissue)	GST (µM/min/mg protein)	PON (µM/min/mg protein)
Control	11.66 ± 0.46	22.66 ± 0.7	639.84 ± 21.62	600.31 ± 13.02
Quercetin	11.31 ± 0.74^{c,d}	24.4 ± 1.39^{c,d}	628.9 ± 42.49^{c,d}	609.26 ± 20.4^{c,d}
Intoxicated	21.26 ± 1.47^{a,b,d}	16.33 ± 0.6^{a,b,d}	446.8 ± 24.98^{a,b}	417.04 ± 16.33^{a,b,d}
Pretreated	17.14 ±1.19^{a,b,c}	20.53 ± 0.74^{b,c}	495.3 ± 32.49^{a,b}	502.24 ± 27.79^{a,b,c}

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p \leq 0.05$ level was set as statistically significant different; (a) significant compared to control, (b) significant compared to quercetin group, (c) significant compared to intoxicated group, (d) significant compared to pretreated group.

Antioxidant system of fetal liver

Moreover, our results showed that maternal exposure to NRDEPs induced a significant decrease of GSH, GST, and PON in hepatic tissues of albino rat fetuses (Table 2).

The observed reduction in GSH level by NRDEPs administration could be attributed to the pro-oxidant activity of NRDEPs, which initiates generation of free radicals / reactive oxygen species (ROS), thus interferes with the endogenous antioxidant system and attributed also to suppression GSH synthesis and/or restriction of intracellular reduction of oxidized GSH [35].

The observed decrease in fetal liver GST activity by NRDEPs in the current study could be attributed to the participated of GST in detoxifying NRDEPs to non-toxic metabolites or by rapidly binding and very slowly turning over these xenobiotics [36,37].

Our results suggest that NRDEPs or its metabolites were responsible for the reduction in PON activity which may act as scavenger for this radical. Otherwise transition metals in NRDEPs might have negative effects on PON activities or on their 3-dimensional structures that are reactive toward thiols forming sulfhydryl-ether linkage [38], and/or able to connect with Zn2+ ion in the active center of PON enzyme and it can diminish the PON activity [39].

Furthermore, the enhanced GSH, GST and PON activities propose that quercetin mediated the modulation of cellular antioxidant levels. These are consistent with previous studies which reported that quercetin can improve the activity of the antioxidant defense system [40] and reduce lipid peroxidation levels [41].

Bax and caspase-3 expression

In the present study, the immunohistochemical results of the fetal liver tissues of the intoxicated group revealed a mild reaction for active caspase-3 (Figure 1C) and moderate reaction for Bax (Figure 1G) in the hepatocytes as compared with both control (Figure 1A and 1E) and quercetin (Figures 1B and 1F) groups that showed a negative reaction for caspase-3 and Bax. However, the liver tissues of albino rat fetuses maternally pretreated with quercetin showed nearly similar to control group and quercetin group for caspase-3 (Figure 1D) and Bax (Figure 1H) reactions.

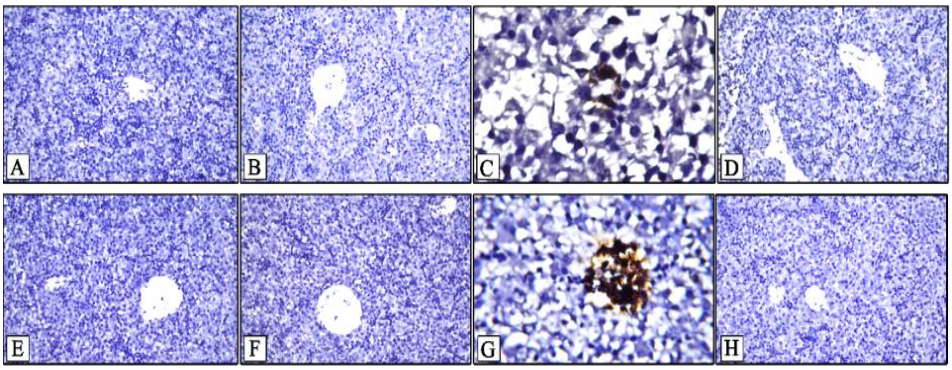

Figure 1: A photomicrograph of caspase-3 (top) and Bax (bottom) immunohistochemical staining of fetal liver sections on the 20th day of gestation; (A,E) control group ×40; (B,F) quercetin group ×40; (C,G) intoxicated group ×80; (D,H) pretreated group ×40

Also, the RT-PCR results confirmed that a significant up-regulation ($P < 0.05$) in the expression level of Bax and caspase-3 genes was observed in both the intoxicated group by 5 and 4.7 folds and pretreated group by 1.6 and 1.57 folds respectively, as both compared with control. However, non-significant down regulation ($P < 0.05$) was observed between quercetin and control groups. Inside out, the pretreatment with quercetin revealed an efficient down-regulation of both Bax and caspase-3 gene expression in fetal liver tissues, (Figure 2).

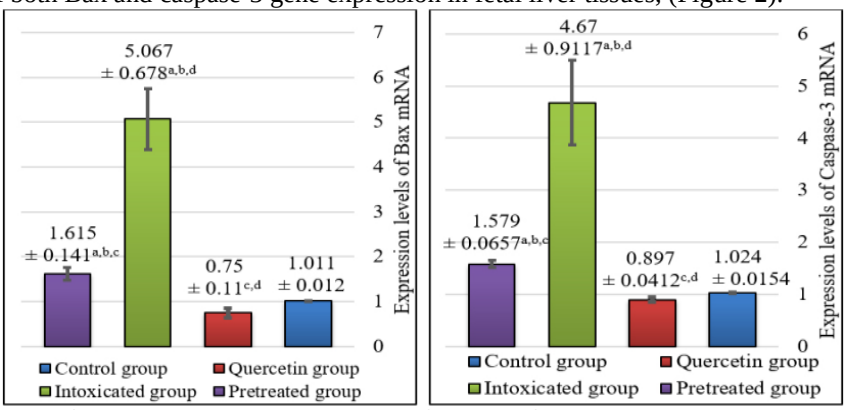

Figure 2: Expression levels of Bax and caspase-3 genes in the liver of albino rat fetuses maternally exposed to the NRDEPs alone and/or pretreated with quercetin

We suggested that the NRDEPs may disturbed the cellular oxidant/antioxidant balance which induces oxidative stress as result from inhibition of the enzymatic and nonenzymatic defenses systems. If this suggestion is valid, NRDEPs will trigger apoptosis via mitochondrial intrinsic pathway through activation of caspase-9 which turn on activation of caspase-3 that initiates the rapid DNA fragmentation.

Apoptosis has two pathways: the intrinsic and the extrinsic pathways. The intrinsic pathway implicates mitochondrial perturbation causing cytotoxicity [42].

The Bcl-2 family consists of anti-apoptotic (e.g., Bcl-2 and Bcl-XL) and a pro-apoptotic (e.g., BH3 and Bax) members, which play an essential role in the controlling of intrinsic pathway of apoptosis [42]. High Bax mRNA expression levels lead to formation of homo- or heterodimers by its ability to interacting with other Bcl-2 members resulted in permeabilization of the outer membrane of mitochondrial [42] which causes disturbance in its transmembrane potential [43]. This lead to release of cytochrome c from mitochondria into the cytoplasm and formation of a complex known as apoptosome [44].

Apoptosome can activate caspase-9 that sequentially activates the effector caspase-3 [45]. Caspase-3 is responsible for destruction of the specific proteins that lead to initiates apoptosis by releasing caspase-activated deoxyribonuclease which triggers rapid DNA fragmentation [46] as described in our study by comet assay.

The liver tissue of albino rat fetuses maternally exposed to 20 mg/kg of NRDEPs significantly increases in the DNA damage estimated parameters (% DNA in tail, % DNA in head, Tail length and Tail moment) compared to those among control and quercetin groups. Contrariwise, pretreatment with quercetin (100 mg/kg) before intoxication by NRDEPs (20 mg/kg) was effective in reducing the DNA damage (Table 3 and Figure 3).

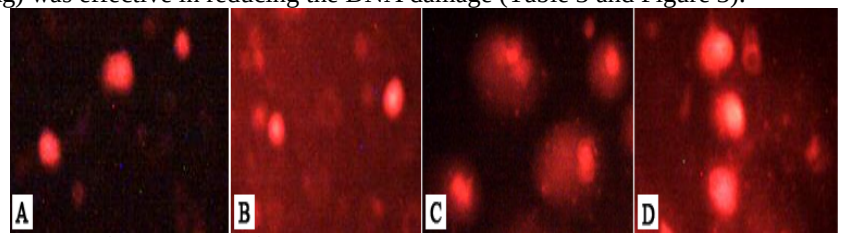

Figure 3: A photomicrograph of comet assay in fetal liver (A) control group; (B) quercetin group; (C) NRDEPs group; (D) pretreated group

Table 3: Effects of NRDEPs and pretreatment with quercetin on comet parameters in fetal liver tissues

	Head DNA (%)	**Tail DNA (%)**	**Tail Length (µm)**	**Tail Moment (AU)**
Control	93.39 ± 0.305	6.61 ± 0.305	3.4 ± 0.167	0.53 ± 0.039
Quercetin	91.22 ± 2.51^{c,d}	8.78 ± 2.51^{c,d}	3.368 ± 0.122^{c,d}	0.56 ± 0.065^{c,d}
Intoxicated	77.18 ± 0.85^{a,b,d}	22.82 ± 0.845^{a,b,d}	5.13 ± 0.267^{a,b,d}	1.37 ± 0.13^{a,b,d}
Pretreated	81.99 ± 1.04^{a,b,c}	18.01 ± 1.04^{a,b,c}	4.2 ± 0.512^{a,b,c}	0.959 ± 0.118^{a,b,c}

Each value represents the mean and SME. One-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) test, the $p \leq 0.05$ level was set as statistically significant different. (a) significant compared to control, (b) significant compared to quercetin group, (c) significant compared to intoxicated group, (d) significant compared to pretreated group.

In the present study, the genotoxicic effect of NRDEPs could be attributed to excessive production of free radicals/ROS and depletion antioxidant enzymes [47,48], Recently, it has been found that, ROS induces double or single stranded DNA breaks, [49,50] and form also oxidative DNA damage products [51]. Another reason of the deleterious genotoxic effects of NRDEPs are believed to be due to excess production of MDA [52], which is a genotoxic byproduct and can binds to DNA [53].This might be the chief cause for augmented severity of DNA damage in fetal liver tissues of NRDEPs treated animals.

Comet assay, in the present study, also confirm anti-genotoxicity and antiapoptotic properties of quercetin in fetal liver tissues as evidenced by effectively down-regulated the high expression of caspase-3 and Bax, which they are often revealing the degree of apoptosis in fetal liver after maternal exposure to NRDEPs and moreover remarkable reduction in the increase of all estimated parameters of DNA damage (Tail length; % DNA in tail and Tail moment) induced by NRDEPs.

The suppression of apoptosis by quercetin may be result from its up-regulation effectively of anti-apoptotic member Bcl-XL [12] and efficiently suppressed of Bax-dependent apoptosis and inhibition of several proapoptotic transcription factors [41].

CONCLUSION

On conclusion, the potent antioxidant quercetin has protective roles against NRDEPs induced oxidative stress along with enhancing antioxidant defense system and inhibiting both Bax and caspase-3 level expression so, it can alleviate apoptosis and DNA fragmentation.

CONFLICT OF INTEREST

The authors declare that they have no competing of interest.

REFERENCES

[1] Mazzarella G, Ferraraccio F, Prati MV, Annunziata S, Bianco A, Mezzogiorno A, Liguori G, Angelillo IF, Cazzola M. *Resp Med*. **2007**, 101(6), 1155-1162.
[2] Loxham M, Cooper MJ, Gerlofs-Nijland ME, Cassee FR, Davies DE, Palmer MR, Teagle DAH. *Environ Sci Technol*. **2013,** 47(8), 3614-3622.
[3] Jung HS, Miller A, Park K, Kittelson DB. *J Air Waste Manage Assoc*. **2013,** 63(10), p. 1199-1204.
[4] Valentino SA, Tarrade A, Aioun J, Mourier E, Richard C, Dahirel M, Rousseau-Ralliard D, Fournier N, Aubrière M-C, Lallemand M-S, Camous S, Guinot M, Charlier M, Aujean E, Al Adhami H, Fokkens PH, Agier L, Boere JA, Cassee FR, Slama R, Chavatte-Palmer P. *Part Fibre Toxicol.* **2016,** 13(1), 39.
[5] Meng Z, Zhang Q. *Environ Toxicol Pharmacol*. **2006,** 22(3), 277-282.
[6] Bourdon JA, Saber AT, Jacobsen KA, Jensen KA, Madsen AM, Lamson JS, Wallin H, Møller P, Loft S, Yauk CL, Vogel UB. Part Fibre Toxicol. **2012,** 9, 5-5.
[7] Danielsen PH, Loft S, acobsen NR J, Jensen KA, Autrup H, Ravanat J-L, Wallin H, Møller P. *Toxicol Sci*. **2010,** 118(2), 574-585.
[8] Fortoul TI, Rodriguez-Lara V, Gonzalez-Villalva A, Rojas-Lemus M, Cano-Gutierrez G, Ustarroz-Cano; L Colin-Barenque M, Montaño LF, García-Pelez I, Bizarro-Nevares P, Lopez-Valdez N, Falcon-Rodriguez CI, Jimenez-Martínez RS, Ruiz-Guerrero ML, López-Zepeda LS, Morales-Rivero A, Muñiz-Rivera-Cambas A. J Biomed Biotechnol. **2011,** 951043.
[9] Iavicoli I, Fontana L, Leso V, Bergamaschi A. *Int J Mol Sci.* **2013,** 14(8), 16732-16801.
[10] Antonio P, Luisa C, Bengt F. *Small*. **2013,** 9(9- 10), 1557-1572.
[11] Poulsen MS, Mose T, Maroun LL, Mathiesen L, Knudsen LE, Rytting E. *Nanotoxicol*. **2015,** 9(sup1), 79-86.
[12] Bu T-l, Jia Y-d, Lin J-x, Mi Y-l, Zhang C-q. *J Zhejiang University SCIENCE B*. **2012,** 13(4), 318-326.
[13] Bradford MM. Analytical Biochem. **1976,** 72(1-2), 248-254.
[14] Yoshioka T, Kawada K, Shimada T, Mori M. *Am J Obstetrics Gynecol*. **1979,** 135(3), 372-376.
[15] Ellman GL. *Archives Biochem Biophysics*. **1959,** 82(1), 70-77.
[16] Habig WH, Pabst MJ, Jakoby WB. J Biol Chem. **1974,** 249(22), 7130-7139.
[17] Gan KN, Smolen A, Eckerson HW, La Du BN. *Drug Metabol Dispos.* **1991,** 19(1), 100-106.
[18] Sati L, Seval-Celik Y, Demir R. *Histochem Cell Biol*. **2010,** 133(1), 85.
[19] Collins AR. Mol Biotechnol. **2004,** 26(3), 249.
[20] Rao X, Huang X, Zhou Z, Lin X. *Biostat Bioinformat Biomath*. **2013,** 3(3), 71-85.
[21] Banni M, Messaoudi I, Said L, El Heni J, Kerkeni A, Said K. *Archives Environ Cont Toxicol.* **2010,** 59(3), 513-519.
[22] Kinouchi S. *Yonago Acta Medica*. **2003,** 46, 109-115.
[23] Shi Y , Song Y, Wang Y, Liang X, Hu Y, Guan X, Cheng J, Yang K. *BioMed Res Int.* **2009,** 2009.
[24] Hougaard KS, Jensen KA, Nordly P, Taxvig C, Vogel U , Saber AT, Wallin H. *Part fibre toxicol*. **2008,** 5(1), 3.
[25] Sugamata M, Ihara T, Takano H, Oshio S, Takeda K. *J health sci*. **2006,** 52(1), 82-84.
[26] Auffan M, Rose J, Bottero J-Y, Lowry GV, Jolivet J-P, Wiesner MR. *Nature Nanotechnol*. **2009,** 4(10), 634.
[27] Andersson H, Lindqvist E, Westerholm R, Grägg K, Almén J, Olson L. *Environ Res*. **1998,** 76(1), 41-51.
[28] Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A. *Environ Health Perspectives*. **2003,** 111(4), 455-460.
[29] Leonard SS, Wang S, Shi X, Jordan BS, Castranova V, Dubick MA. *Toxicol.* **2000,** 150(1), 147-157.
[30] Sørensen M, Loft S, Andersen HV, Raaschou-Nielsen O, Skovgaard LT, Knudsen LE, Nielsen IV, Hertel O. *J Exposure Anal Environ Epidemiol.* **2005,** 15, 413.
[31] Tu B, Liu Z-J, Chen Z-F, Ouyang Y, Hu Y-J. *RSC Advances*. **2015,** 5(128), 106171-106181.
[32] Ross JA, Kasum CM. Annual review of nutrition. **2002,** 22(1), 19-34.
[33] Rice-Evans CA, Miller NJ, Paganga G. *Free radical Biol Med*. **1996,** 20(7), 933-956.
[34] Bakheet SA. *Oxidative Med Cell Longevity*. **2011,** 2011.
[35] Mohan D, Thiyagarajan D, Murthy PB. Afr J Pharm Pharmacol. **2013,** 7(7), 318-331.

[36] Jebur AB, Nasr HM, El- Demerdash FM. *Environ Toxicol*. **2014,** 29(11), 1323-1329.
[37] Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A. Neurochem Int. **2014,** 78, 122-129.
[38] Nishio E, Watanabe Y. Biochem Biophy Res Comm. **1997,** 236(2), 289-293.
[39] Demir N. 2011.
[40] Papiez MA, Cierniak A, Krzysciak W, Bzowska M, Taha HM, Jozkowicz A, Piskula M. *Food Chem Toxicol*. **2008,** 46(9), 3053-3058.
[41] Attia SM. *Mutagenesis*. **2010,** 25(3), 281-288.
[42] Danial NN, Korsmeyer SJ. *Cell***. 2004,** 116(2), 205-219.
[43] Kroemer G, Galluzzi L, Brenner C. *Physiol Reviews*. **2007,** 87(1), 99-163.
[44] C-X Lü, Fan T-J, Hu G-B. Cong R-S. Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica. **2003,** 35(10), 881-885.
[45] Johnson CR, Jarvis WD. *Apoptosis.* **2004,** 9(4), 423-427.
[46] Han B, Wang Q, Cui G, Shen X, Zhu Z. Neurochem Int. **2011,** 58(2), 224-233.
[47] Hiura TS, Li N, Kaplan R, Horwitz M, Seagrave J-C, Nel AE. J Immunol. **2000,** 165(5), 2703-2711.
[48] Tseng C-Y, Wang J-S, Chang Y-J, Chang J-F, Chao M-W. Cardiovascular Toxicol. **2015,** 15(4), 345-354.
[49] Gustafsson ÅB; Gottlieb RA. *Am J Physiol-Cell Physiol*. **2007,** 292(1), C45-C51.
[50] Shen B, He P-J, Shao C-L. *PLoS One*. **2013,** 8(12), e84610.
[51] Meseguer M, Martínez-Conejero JA, O'Connor JE, Pellicer A, Remohí J, Garrido N. *Fertility and Sterility*. **2008,** 89(5), 1191-1199.
[52] Risom L, Møller P, Loft S. *Mutat Res*. **2005,** 592(1), 119-137.
[53] Eder E, Wacker M, Lutz U, Nair J, Fang X, Bartsch H, Beland FA, Schlatter J, Lutz WK. *Chemico-Biol Interact*. **2006,** 159(2), 81-89.